Test Plan for the Performance Evaluation of the Siemens SiCURE Ballast Water Management System

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1. MERC and GSI Background and Objectives

The Maritime Environmental Resource Center (MERC) is a State of Maryland initiative that provides test facilities, information, and decision tools to address key environmental issues facing the international maritime industry. The primary focus is to evaluate the mechanical and biological efficacy, costs, and logistical aspects of ballast water treatment systems and to assess the economic impacts of ballast water regulations and management approaches. A full description of MERC structure, products, and services can be found at www.maritime-enviro.org.

To address the need for effective, safe, and reliable ballast water treatment systems to prevent the introduction of non-native species, MERC has developed as a partnership between the Maryland Port Administration (MPA), Chesapeake Biological Laboratory/ University of Maryland Center for Environmental Science (CBL/UMCES), U.S. Maritime Administration (MARAD), National Oceanic and Atmospheric Administration (NOAA), Smithsonian Environmental Research Center (SERC), and University of Maryland (UM) to provide independent performance testing and to help facilitate the transition of new treatments to operations. Treatment evaluation efforts will also take advantage of expertise and the rigorous technology evaluation format/process developed by the Alliance for Coastal Technologies (ACT, www.act-us.info). ACT is NOAA-funded distributed testbed, headquartered at CBL/UMCES, dedicated to fostering the development and adoption of effective and reliable sensors for studying and monitoring coastal environments.

The Great Ships Initiative (GSI) is a collaborative not-for-profit endeavor to resolve barriers to effective and efficient ballast water treatment by ships. To that end, GSI evaluates the performance characteristics of proposed ballast water treatment systems at the bench-, land-based and shipboard scales. GSI land-based and shipboard testing is reserved for operationally feasible treatment systems likely to meet prevailing performance standards and environmental soundness requirements. The goal of GSI ballast treatment research services at the land-based and shipboard scales is to provide shipping lines, treatment developers and regulators with an independent and credible assessment of treatment performance under realistic freshwater challenge conditions. To that end, the fundamental approach of GSI is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of ballast water treatment systems under challenging ambient freshwater conditions based on the International Maritime Organization (IMO) G8 guidelines and the U.S. Coast Guard supported ETV protocols under development.

The following protocols describe how MERC and GSI will evaluate the performance characteristics of the Siemens Water Technologies SiCURE^{TM} Ballast Water Management Systems through objective and quality assured land-based testing (dockside at a flow rate of 200m^3/hr). The goal of this specific evaluation is to provide shipping lines, regulators, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the data and information on performance characteristics will cover legitimate information that users need and will compare performance against the International Maritime Organization (IMO) D2 regulatory discharge standards.

It is important to note that MERC and GSI themselves do not certify technologies or guarantee that a treatment will always, or under circumstances other than those used in testing, operate at the levels verified. Treatment systems are not labeled or listed as acceptable or unacceptable but tests and presented results are in a format consistent with that requested by

specific regulations (e.g., IMO D2, G8 and G9) so that can be used to determine regulatory compliance by appropriate agencies of certification societies. Final reports on technology performance will be reviewed by the MERC and GSI Advisory Board/Committee and provided to Siemens and the MERC/GSI funding agencies prior to public release. All specific terms of a testing program associated with a particular treatment system, including management of test findings, are outlined in a Participation Agreement executed between the treatment developer and MERC and the Northeast Midwest Institute, the GSI managing entity.

2. Treatment to be Evaluated

Siemens Water Technologies has developed the SiCURE™ Ballast Water Management Systems (BWMS) based on the maritime industry proven Chloropac® Electrochlorination system for ship's cooling water piping. This system was first developed in the early 1970s and has been in operation onboard over 2,000 vessels.

SiCURE has several unique features designed to provide effective treatment of ballast water while minimizing risk to the environment, the ship, and its crew. SiCURE is based on electrolysis of seawater and use of hypochlorite as an Active Substance at a viable, "meet the demand" dose. The system injects only as much Active Substance into ballast water as required to achieve the necessary level of disinfection. This approach is aimed at eliminating over chlorination and associated risks of corrosion and generation of disinfection by-products.

3. Overview of Test Facilities

Basic Approach:

The specific protocols described below are based on the IMO G8 guidelines and the US Coast Guard supported ETV protocols under development. The fundamental approach of MERC and GSI is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of ballast water treatment systems. Therefore, MERC and GSI rely on challenging ambient conditions found in the Chesapeake Bay and Duluth-Superior Harbor, and do not artificially augment test waters in most evaluations, to avoid artifacts and the potential to overestimation of system performance (see Table 1). For example, rapid changes in physical conditions (such as salinity or total suspended solids) as ambient organisms are being brought in with ballast water may cause significant mortality, independent of treatment. Similarly, concentrating natural assemblages of plankton on nets, and introducing them into ballast water being pumped into tanks, can often result in significant handling associated mortality. Given the unpredictable physical and biological conditions found in all natural waters, IMO G8 MEPC 58/23 ANNEX 4, Part 2, Section 2.3.36 is used by MERC and GSI as the standard for a valid test trial: "If in any test cycle the average discharge results from the control water is a concentration less than or equal to 10 times the values in regulation D-2.1, the test cycle is invalid". While a goal of MERC and GSI is provide independent G8/ETV data on the performance of ballast water treatment systems, it is ultimately up to an Administration to decide if the system meets their requirements for Type Approval Certification.

Table 1. Ranges of various physical and biological parameters in ambient water during the testing season (March/April – October/November) in the Port of Baltimore in comparison to ETV/USCG and IMO G8 recommended challenge conditions. Port of Baltimore data collected by MERC and various academic and agency studies or monitoring efforts in the general location of the *Cape Washington* (Patapsco River). Ranges in various physical and biological parameters of ambient water during the testing season (July – September) in the Duluth/Superior Harbor collected by GSI.

Parameter	Proposed ETV/USCG†	Recommended IMO G8‡	Historic Ranges* Port of Baltimore	Historic Ranges◊ Duluth/Superior Harbor
Temperature (^{o}C)	10 - 35	–	4 - 28	9 - 22
Salinity (psu)	0 - 31	Two salinities, >10 psu difference	5 - 15	0 - 1
Total Suspended Solids (mg/l)	> 15	> 50	1 - 60	2 - 21
Particulate Organic Carbon (mg/l)	> 1	> 5	0.5 - 6.0	TBD
Dissolved Organic Carbon (mg/l)	> 3	> 5	2 - 10	6 - 22
Zooplankton (> 50 μm) / m^3	> 10,000	> 100,000	10,000 - 300,000	10,000 - 3,000,000
Phytoplankton (10 - 50 μm) / ml	> 100	> 1,000	500 - 10,000	25 - 500
Heterotrophic Bacteria cfu / ml	> 1,000	> 10,000	10,000 - 10,000,000	5,000 - 15,000

† Generic Protocol for the Verification of Ballast Water Treatment Technologies: Draft v4 2008, US EPA Environmental Technology Verification (ETV) program under contract to US Coast Guard.
‡ IMO Guidelines for the Approval of Ballast Water Management Systems (G8), October 2008, Annex 4 Resolution MEPC.174(58).
* TSS, POC and DOC (2004-2007) MD DNR Chesapeake Bay Water Quality database: www.chesapeakebay.net/data_waterquality.aspx. Zooplankton (1998 – 2002) and phytoplankton (2004-2007) Chesapeake Bay Program: www.chesapeakebay.net/data_plankton.aspx. Bacteria (1998 – present) Cowell and Huq, University of Maryland; Louis et al. 2003, AEM 69:2773-2785.
◊ Values collected by GSI in 2007 and 2008 as part of facility validations and treatment systems testing www.nemw.org/GSI/.

For this specific evaluation of the SiCURE treatment system, Siemens Water Technologies has made a special request to MERC and GSI to augment intake water to more consistently approach the initial challenge water conditions described in the G8 guidelines during the test trials. While MERC and GSI do not make any guarantees on the precise conditions of challenge water, natural/local harbor sediments and/or humic acid (as proposed by ETV and NRL Key West) will be injected inline during initial filling of control and test tanks at MERC and GSI to increase TSS, POC and DOC levels, and algae will be added inline to water during filling of control and test tanks at GSI. Details on these processes are available upon request and will be provided in the final report.

Summary of MERC Land-Based Facility and Sampling Design:

MERC will evaluate the biological efficacy of the SiCURE ballast water management system onboard the MARAD vessel M/V *Cape Washington* while docked in Baltimore Harbor, Maryland (right). The ballast system of the *Cape Washington* has been modified to allow for water at a flow rate of 400m^3/hr to be split equally, and delivered simultaneously, to a "control" (untreated) ballast tank and a "test" (passing first through the SiCURE system) ballast tank, each at 200m^3/hr. The ship's ballast tanks to be used for the required holding time of five days are essentially identical in size (~ 650 m^3) and structure. Each tank will be filled to approximately 250 m^3 for test trials. A detailed drawing of the modified ship ballast system can be found on page 24.

Care was taken in the design of the MERC *Cape Washington* test systems so that water entering the control and test tanks is handled (e.g., passing through same pump and similar piping) as close to identical as possible, aside from passing through the SiCURE system for treatment. Three test system performance runs have been conducted to assure that water in both control and test tanks have near identical physical and biological conditions. While initial physical and biological conditions are subject to natural variability, the MERC test system itself is not a source of mortality (data available upon request). The test ballast tank will also drained and manually rinsed/cleaned prior to conducting the first evaluation trial, and rinsed/flushed with 20 – 30 m^3 of potable water and drained completely between trials, to avoid the possibility of residual live organisms in the bottom of the empty test tank influencing results.

Five sequential samples will be taken for each of the following: (A) initial/intake conditions, just prior to the split of control and treated water, (B) initial conditions just downstream of the SiCURE system during filling of test tank, (C) control water upon discharge after a five-day holding time, and (D) treated water upon discharge after a five-day holing time. Sample volumes and details of the physical, chemical, and biological analyses for each sample are described below. A detailed drawing of the MERC *Cape Washington* test setup and sampling design is available on page 25.

All samples collected to quantify live organisms or water quality will be taken by inline sampling of ballast water during the initial filling or during discharge of water from the ship's tanks by sample ports place in appropriate filling or discharge pipes. All sample ports include a valve and sample tube with a 90^o bend towards the direction of flow, placed in the center of the piping system (based on the design developed and validated by the US Naval Research Laboratory, Key West Florida).

A total of 10 identical conical bottom mesocosms (shown below) have been installed on the *Cape Washington* to allow for precise and controlled sampling during each test trial. Five replicate mesocosms are used to sample initial, challenge conditions at the start of each trial, prior to the split in water to control and test tanks. The second five mesocosm are used to sample after water has passed through the SiCURE treatment during the initial filling of the test tank. At the end of each trial (after five-days), five mesocosms are used for sampling water from the

control tank, and the second five mesocosm for water from the test tank. At each sampling time (initial and after holding time), the designated five mesocosms will be filled to approximately 1.05 m^3 in sequence over 75 to 80 minutes of the 90 minutes required to fill or drain the ship's ballast tanks (i.e., sampling takes place > 80% of the time during filling or draining of tanks). Immediately after filling of each mesocosm (< 5 minutes), physical parameters of the water will be measured (see below), and then the precise samples volumes described below will be collected for each biological and water quality categories by gravity draining through a bottom valve and tubing. A table (Table 2) of samples to be collected, with corresponding volumes and purpose can be found on page 27.

Each mesocosm has been calibrated (by filling with potable water and a flow meter) and marked with known volumes to assure accurate sample collection. Each mesocosm will also be rinsed thoroughly with potable water for a minimum of three times after each use and kept clean and dry between uses.

MERC test and sampling system on the *Cape Washington*.

Summary of GSI Land-Based Facility and Sampling Design:

GSI evaluates the biological efficacy ballast water treatments at a purpose-built, land-based ballast treatment test facility located in the Duluth-Superior Harbor of Lake Superior (right). The facility draws raw intake water from Duluth-Superior Harbor at up to 680 m^3/hr. A Y-split in the intake piping simultaneously channels one half of the flow (up to 340 m^3/hr) to a treatment track and one half (also up to 340 m3/hr) to a matched control track. The treatment track directs water through the experimental treatment system and into a 200 m^3 cylindrical retention tank. The control track by-passes the treatment system and channels water directly into a matched control retention tank. After storage, water is discharged sequentially from the treatment and control tanks at m^3/hr to the harbor or wastewater treatment facility. A detailed design can be found on page 26 and information on GSI Facility Validation can be found at www.nemw.org/GSI/.

Water is sampled continuously throughout ballasting functions (intake or discharge) through in-line sample points. There are 14 in-line sample points at the GSI land-based facility in total, though not all are in use at this time. Each sample point is made up of three identical sample ports with a center-located elbow-shaped pitot tube (90^o) bent towards the direction of water flow used to sample the water. This pitot design is based on a design developed and validated by the U.S. Naval Research Laboratory in Key West Florida, and empirically at GSI. Intake sampling uses sample ports (page 26) at the paired intake sample points of SP#2 and SP#3 on the control and treatment tracks for concurrent sample water collection. Discharge sampling uses sample ports at the discharge sampling points of SP#9 and SP#10 (page 26), with sequential collection of control and treatment water.

Sample water at a given sampling location (i.e., intake line of the control track, intake line of the treatment track, or the discharge line for the control or treatment tracks) is transferred simultaneously and continuously throughout ballasting operations (intake or discharge) from at least two of three replicate in-line sample ports to at least two of three replicate 3.8 m^3 sample collection tubs via a 3.8 cm ID PVC transfer pipe and an automated pressure-controlled diaphragm valve.

Well-mixed time-integrated 1 l whole water phytoplankton, microbial and water quality samples are immediately extracted from the replicate sample collection tubs, with the remainder of the collection tub sample concentrated through 35 µm plankton nets to retain all zooplankton. Each replicate intake sample (control and treatment tracks) is time-integrated over the 45 minute fill period and at least 1 m^3 in volume. Each replicate discharge sample is time-integrated over the 45 minute discharge period and at least is 1 m^3 in volume for control track samples and up to 3 m^3 in volume for treatment track samples. On intake, control and treatment track samples are collected simultaneously. On discharge, they are collected sequentially, but within two hours of

each other. A table (Table 2) of samples to be collected, with corresponding volumes and purpose can be found on page 27.

Live analysis of zooplankton occurs on-site within one hour of filtration through the plankton net. Live analysis of phytoplankton samples occurs on-site within 1.5 hours of sample collection. All filtered or whole water samples are stored in coolers until they can be analyzed. Microbial samples, including spiked MS2 bacteriophage samples, are transported immediately following collection in an insulated container for analysis at the University of Wisconsin-Superior laboratory, located within 10 minutes drive of the land-based facility.

An on-site mobile field laboratory provides bench-scale facilities to support time sensitive assays associated with the GSI land-based tests, including live analysis of phytoplankton and zooplankton (right). The laboratory is climate-controlled, and has enough desk and counter space to allow for simultaneous microscopic and analytical analysis of samples.

4. Test Trials

Each facility (MERC and GSI) will conduct a maximum of six test trials (12 total) of the SiCURE system to assess its ability to meet IMO D2 ballast water discharge standards in land-based testing during the spring/summer 2009. As noted above, a valid test is regarded as one for which discharge densities of live organisms are at least 10 times the IMO D2 standard, consistent with IMO G8 MEPC 58/23 ANNEX 4, Part 2, Section 2.3.36.

Two treatment calibration test runs for the SiCURE system will also be allowed just prior to the formal evaluation at each facility. For any test that is considered valid (and for which the facility testing system functioned properly), an inability to: (a) successfully treat ballast water without interruption, (b) to meet D2 discharge standards after a five-days holding time, and/or (c) to discharge water environmentally benign (i.e., no residual toxicity) water (see page 12), will be considered a "failure". Results of tests regarded as failures will be noted and included in the final report. Two failures on the part of the SiCURE system may result in the termination of testing prior to the maximum of six test trials depending on the nature of the failures. MERC and GSI Senior Management will make a final decision on early termination of the tests, in consultation with Siemens staff.

This evaluation will be based on physical and biological characterization of water upon ballasting (uptake of water) and comparisons of organisms in control versus treated water after a five-day, in-tank holding time for the different D2 biological categories. Results will also be presented as concentration of viable organisms per biological category in treated water upon discharge versus IMO D2 standards.

MERC and GSI have worked to standardize methods and approached (described below) to evaluate the performance of ballast water treatment systems. Some subtle difference may exist as a result of specific facility design and estuarine versus fresh water and associated organism. However, these do not compromise the scientific rigor or comparability of results

between the two sets of tests. The following sections describe how each parameter and variable is sampled/analyzed and additional details can be found in Appendices and at www.nemw.org/GSI/SOPS.htm.

5. Methods

Quantifying Physical Conditions:

Temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity and pH will be measured every 15 minutes during the test trials by two identical multi-parameter probes (calibrated according to manufactures specification) placed, one each, into the control and test tanks. A third hand-held instrument will be used to measure temperature, salinity, and dissolved oxygen of water in each replicate sample (described above) as it is collected.

Initial inline samples (three replicates, 500 ml - 2 l each) of ballast water during the filling of the control and test tanks will also be collected, filtered, and analyzed for the water quality parameters of particulate organic carbon (POC), dissolved organic carbon (DOC), and total suspended solids (TSS). See Appendices A, B and C for details.

Quantifying Viable Organism > 50 µm in size:

As described above, MERC uses five 1 m^3 mesocosms (a 5 m^3 integrated sample) and GSI use three 1 m^3 mesocosms (a 3 m^3 integrated sample) to sample each time point and treatment type (Table 2, page 27). Sampling occurs during initial uptake of water, just downstream of the treatment systems during filling of the test tank, and upon discharge of control and treated water (after 5 days). Immediately after filling, each mesocosm will be drained through a 35 µm (50 µm diagonal dimension) plankton net to concentrate the zooplankton for examination under a dissecting microscope. The proportion and total concentration of live versus dead organisms will be determined using standard movement and response to stimuli techniques and this live/dead analysis will take place within one hours of collecting the individual samples. Depending on concentrations, quantification of zooplankton in initial samples (upon ballasting) and control samples may require analysis of sub-samples and extrapolation to the entire 1 m^3. Zooplankton samples will then also be fixed with buffered, 10% formalin in 125ml Nalgene bottles and shipped to the SERC for additional taxonomic evaluations. Total counts and general taxonomic classification will be conducted under a dissecting microscope at 25X, except for some taxa, which will be removed and identified using a compound microscope. Larval forms of invertebrates will be identified to higher taxonomic levels such as order (e.g., Decapoda) suborder (e.g., Balanomorpha) or class (e.g., Bivalvia). Adults will be identified to species in most cases.

Quantifying Viable Organism 10 - 50 µm in size:

MERC - Two liters of unfiltered water for each mesocosm (a 10 l integrated sample) will be collected immediately after filling, to determine concentrations of organisms in this size class using four distinct methods (A – D below, Table 2 page 27). All samples will be held in amber Nalgene bottles and transported on ice to laboratories where analyses occur within 3 hours of collection. (A) One sub-sample from the initial 2 l will be fixed with standard Lugol's solution, and placed in a 250 ml amber Nalgene bottles to determine total cell abundances under an inverted compound microscope using grid settlement columns and phase contrast lighting. (B) A

second 250 ml sub-sample will be stained using CMFDA (5-chloromethylfluorescein diacetate) as a selective live/viable indicator. Samples stained with CMFDA, are incubated and observed on a Sedgewick Rafter slide using a Leitz Laborlux S modified for epifluorescence. Cells are scored as live when showing strong fluorescence signature under excitation (some cells also showed motility). However, it is also widely accepted that these direct count and staining techniques have limitations (Lugol's does not selectively stain live or dead, various algal species take up CMFDA differently, and other particles in a sample can fluoresce). Therefore, analyses of chlorophyll are also conducted as supporting information. (C) A third sub-samples is filtered (Whatman GF/F 0.7 μm pore, 2.5 cm diameter membrane) and frozen (-80^{o}C) until analysis of total active chlorophyll-a by the CBL/UMCES Nutrient Analytical Services Laboratory using US EPA Methods 445.0 for extractive/fluorometric techniques (see Appendix D). (D) Finally a fourth sub-sample is used to determine chlorophyll levels after allowed to regrow under favorable conditions. Algae specific vitamins, minerals, and nutrients (Guillard 1975, F/2 formulation) are added to a sub-sample from each mesocosm and are placed in a standard algal culture light-dark regimen for six days, prior to extractive chlorophyll-a analysis. An increase in chlorophyll, or positive regrowth, indicates that viable phytoplankton were in the samples, whereas chlorophyll levels at or below detection limits of the laboratory analytical method suggests that there was no viable phytoplankton. Although precise abundances of cells/ml cannot be determined for diverse communities of phytoplankton using these types of regrowth experiments, this is a conservative method used to determine the presence/absence of living organisms.

GSI - For live analysis of organisms 10 – 50 μm in size at least 1 l of unfiltered water is taken from each of the triplicate control and treatment sample collection mesocosm/tub (a 3 l integrated sample, Table 2 page 27). Analysis occurs on-site within 1.5 hours of sample collection, with samples stored in coolers during the interim. Prior to analysis, samples are concentrated through a 10 μm plankton net and stored in a 25 ml sample container. Next, a 1.5 ml subsample is transferred to a 2-ml sample container, with 4 μl of FDA stock solution added. The subsample is then allowed to incubate in the dark for 5 minutes. For analysis, the concentrated algae sample is mixed and immediately transferred to a Sedgwick-Rafter cell, covered and placed on the stage of microscope that is set for simultaneous observation using brightfield and epifluorescence. At least 100 entities are then counted and identified along the horizontal transects, aiming for at least 100 entities (i.e., unicellular organism, colony or filament). Single cell entities and cells comprising colonial and filamentous entities are characterized as follows: alive = cells showing obvious green fluorescence from cell contents; dead = cells showing no or very little evidence of green fluorescence from cell contents (note: for entities containing multiple cells, all cells must be confirmed as dead to fulfill this category); and ambiguous = entities that cannot be clearly identified as alive or dead (should be uncommon). Entities that are less than 10 μm in all visible dimensions or greater than 50 μm in minimum dimension are not counted. Records are kept of transect lengths and widths so that the total counted area may be calculated later. Counting and measurement of entities follows standard procedures for individuals (length and width), colonies (e.g., number of cells, cell length and width) and filaments (e.g., number of cells, cell length and width or total filament length if cells cannot be discerned). The remaining concentrated sample in the 25 ml bottle is archived using a preservative (formalin or Lugol's) for long-term storage.

Quantifying Viable Indicator Pathogens:

A 1 l sample of water for each mesocosm/tub (a 5 l integrated sample for MERC and a 3 l integrated sample for GSI) is collected to determine concentrations of total heterotrophic bacteria and three specific indicator pathogens, *E. coli*, intestinal *Enterococci*, and toxigenic *Vibrio cholerae* (Table 2 page 27). Total heterotrophic bacteria are enumerated by spread plate method using NWRI agar according to *Standards Methods for the Examination of Water and Wastewater* (21st edition, 2005). The presence and abundance of *E. coli* and intestinal *Enterococci* is determined using a commercially available chromogenic substrate method (IDEXX Laboratories, Inc.; Noble et al. 2003) and 10 ml and 100 ml water sample aliquots. Additionally, concentrations of culturable *E. coli* and intestinal *Enterococci* are determined using a standard USEPA method, namely, membrane filtration on mTEC agar (*E. coli*) (1 ml, 10 ml and 100 ml) and mEA agar (*Enterococcus*) (10 ml and 100 ml). Abundance of total and toxigenic *V. cholerae* are calculated by filtration and selection on TCBS agar and enumerated using species-specific RNA colony blot (500 µl to 1 ml) and *ctxA* DNA colony blot (1-10 ml). Viable toxigenic *V. cholerae* is assayed with a commercial DFA kit specific for serogroup O1 (New Horizons Diagnostics) using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) (Hasan et al. 1994).

Data Analysis:

Although multiple mesocosms, samples, and measures from each tank will be taken, to avoid pseudo-replication, the unit of replication for statistical analyses is each trial (n = 5 or 6). We assume that all measures for a single trial provide one estimate of treatment efficacy. Thus, treatment efficacy for any biological parameter is estimated as changes found before and after trial (percent reduction), and as the difference in concentration between treated water and IMO standards. This approach controls for variation due to temporal changes in environmental conditions.

6. Protocols for Evaluations of SiCURE System Discharge Toxicity

MERC - The MERC Testing Team members at the University of Maryland Wye Research and Education Center (WREC) will evaluate the aquatic toxicity of the ballast water discharge. The testing is designed to meet Section 5.2 of the Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9) as resolved by the Marine Environmental Protection Committee of the International Maritime Organization (IMO, 2008). Section 5.2 states that, "The advantage of conducting toxicity testing on the ballast water discharge is that it integrates and addresses the potential for interactions of the Active Substances and Preparations with the possible by-products." This section requires that, "these toxicity tests should include chronic test methods with multiple test species (a fish, an invertebrate and a plant) that address the sensitive life-stage. The preference is to include both a sub-lethal endpoint (growth) and a survival endpoint." The MERC approach to meet these IMO guidelines use test methods and species employed by the EPA for Whole Effluent Toxicity (WET) testing of effluents. These methods are approved by the EPA (2002) and the American Society for Testing and Materials (ASTM, 2006). Personnel at WREC are vary familiar with these test species and methods and conducted WET testing from 1986 to 2003 for the Maryland Department of the Environment in support of its NPDES WET bioassay-monitoring program.

Test Species:

A fish, an invertebrate and a plant (algae) will be used in all ballast discharge tests. Because the test site in Baltimore Harbor is a mesohaline aquatic environment with salinities ranging from 5 to 15 psu, estuarine organisms will be used in these tests. The algal species will be *Isochrysis galbana* or *Tetraselmis suecica* depending on which species performs best in preliminary testing. The algae will be purchased from the University of Texas Algal Culture, University of Texas, Austin, Texas. The growth media for these species will be those given in Appendix A3 of ASTM Designation E 1218-04 "*Standard Guide for Conducting Static Toxicity Tests with Microalgae*" (ASTM, 2006). The culture conditions will follow those given in this guide. The fish species used in the test will be the sheepshead minnow (*Cyprinodon variegatus*) while the invertebrate species will be the mysid (*Americamysis bahia*; formerly *Mysidopsis bahia*). These are estuarine test species suggested for use in EPA's Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms (EPA, 2002). Test organisms will be purchased from Aquatic BioSystems of Fort Collins, Colorado. This company is our regular supplier of test organisms. They provide excellent QA/QC, including reference toxicant testing and quality control charts for all of their test species. Upon receipt by WREC, holding of test organisms will be conducted in accordance to guidelines outlined in the above referenced EPA manual (2002).

Active Substance and Measurement:

The test solution will be ballast water discharged from the test tanks during each trial of the SiCURE system. The active substance involved in this treatment is chlorine. According to Section 5.2.8 of the IMO G9 resolution, information on Total Residual Oxidants (TRO) and Total Residual Chlorine (TRC) should be provided as part of the application for evaluation, for both the ballast treatment process and the ballast water discharge. The Standard Methods for the Examination of Water and Wastewater Low-Level Amperometric Titration methods 4500-Cl D and E will be used to measure TRO and TRC in the ballast water discharge and in the various test dilutions. A Fischer and Porter amperometric titrator (Model 17T2000) will be used for all measurements. By using the high-sensitivity mode, a forward titration, and a 200 ml sample, TRO quantification limits for method 4500-Cl D are 15 µg/L TRO. With this sample size, 1 ml phenylarsene oxide (PAO, 0.00564 N) titrant equals 1 mg/L chlorine equivalents. For lower levels of oxidant, method 4500-Cl E will be used. A fourfold-diluted PAO titrant (0.00141 N) and a strip-chart recorder for signal amplification from the Fischer and Porter amperometric titrator (Model 17T2000) will be used to measure TRO concentrations to 5 µg/L. Samples will be analyzed immediately upon collection onboard the *Cape Washington* to avoid loss of oxidant due to holding. In addition to the amperometric titration method we will use a YSI Mulitmeter (Model # 556) equipped with a probe to measure oxidation reduction potential (ORP). The probe uses a platinum button sensor giving the instrument a range of -999 to +999 mV, an accuracy of ± 20 mV and a Resolution of 0.1 mV.

Experimental Design and Test Conditions:

Toxicity tests will be conducted on the discharge from all test trials. As required by the IMO G9, the discharge water will be tested with three estuarine species as described in Section 2.1. Both acute and chronic data will be generated for each test. A dilution series, using Baltimore Harbor water, will be run for each species.

Test samples will be collected at the time of discharge from the MERC facility. Samples will be collected by the MERC staff for analysis of both the efficacy of treatment at eliminating organisms from the ballast water and to investigate residual toxicity at discharge as described above. For the suite of toxicity tests, a volume of 38 L (10 gallons) must be collected. This includes enough water to do all of the test renewals. Test water will be stored in large HDPE containers and held at 4°C in the dark to retain as much of the initial toxicity as possible. Portions of this sample will be used each day to serve as the renewal water for the bioassay. Sub-samples of each will also be sent to a certified chemistry laboratory (TBD) for analysis of disinfection byproducts. MERC Testing Team will collect/deliver all samples for chemical analysis and manage analytical results but costs of chemical analysis will be covered by Siemens.

Summaries of the proposed test methods are given in Tables 4 through 8 (page 28). All of the tests will be conducted at the WREC toxicology laboratory. Since chlorine degrades rapidly, all toxicity tests will be initiated within two hours of the completion of a specific trial. Pilot studies have demonstrated that there is no measurable difference in chloronated water held for five days and then either tested within 30 minutes of collection or after a two hour holding and transport time. Standard EPA (2002) and ASTM (2006) methods that have used in at WREC since 1987 to conduct Whole Effluent Toxicity tests and single compound toxicity tests, will be employed. The survival and growth end-points from these tests are those required by the G9 document in Section 5.2.4 (IMO, 2008). The algae test represents a true population growth test.

In addition to the ballast water discharge efficacy testing, sampling and toxicity testing of the water in the test tank will also be conducted on a minimum of one of the six test trials. Sampling will be done at the time of tank filling/treatment, and at one day and five days. The analytical and bioassay methods described above will be used to analyze these different time point samples.

Statistical Analyses:

Toxicity endpoints will include survival in acute fish and invertebrate tests, survival and growth in chronic fish and invertebrate tests, and population growth in chronic algal tests as required in Section 5.2.4 of the G9 (IMO, 2008). Tests are designed with a dilution series to allow calculation of daily LC50 (concentration yielding 50% lethality) values from acute and chronic mortality data. In addition, chronic tests will include sufficient treatment replication to allow calculation of NOEC (no observable effect concentration), LOEC (lowest observable effect concentration) and EC25 (percent concentration yielding a 25% effect) values for all toxicity endpoints as required in Section 5.2.5 of the G9 (IMO, 2008). Statistical analyses will be performed using ToxCalc statistical software (TSS, 2006) according to methods from USEPA (2002) and ASTM (2006) guidance documents. Briefly, LC50s at daily intervals will be calculated from survival data using the Probit Method if an adequate dose response is achieved. If an adequate dose response is not achieved (e.g., only one partial mortality between the concentration causing 100% mortality and that causing 0% mortality), the Trimmed Spearman-Karber Method will be used. Chronic data will be tested using a Probit Method (EC25) and by analysis of variance (ANOVA) with means testing (NOEC/LOEC). Prior to ANOVA testing chronic data will be tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using the Bartlett's Test. Survival data will be arcsine square root transformed prior to analysis. If normally distributed and homogeneous survival and growth data will be analyzed

using a one-tailed ANOVA followed by a Dunnet's means comparison test (equal number of replicates/treatment) or a T-Test with Bonferroni Adjustment (unequal replicates/treatment) to determine differences from control data. If data do not pass the assumptions of normality or homogeneity, a Steel's Many-One Rank Test (equal replicates) or Wilcoxon Rank Sum Test with Bonferroni Adjustment (unequal replicates) will be performed. A *p* value of 0.05 will be used for all hypothesis tests; a *p* value of 0.01 will be used for testing assumptions of normality and homogeneity of variance. Results from the chronic statistical analyses will provide NOECs, LOECs, and EC25s for each ballast water treatment run.

Definition of Test Failure on the Grounds of Toxicity:

Permissible residual toxicity will follow the guidelines outlined by the EPA National Pollutant Discharge Elimination System (NPDES) for issuance of a Vessel General Permit (VGP) (full text is available at www.epa.gov/npdes/vessels; relevant sections on ballast discharge toxicity are 5.8.1.2 and 15.2). Based on these criteria a test trial will be considered a failure on the grounds of residual toxicity upon discharge if acute lethality (as indicated by determination of an LC50 of less than 100%) occurs in any test species. Determination of test failure as a result of chronic toxicity will be based on EC25 analyses. An EC25 is a point estimate of the toxicant concentration (expressed as percent effluent) that causes an observable adverse effect in 25 percent of test organisms. Chronic test results will be calculated in TUc (chronic toxicity units), where TUc = 100/EC25 (e.g., an EC25 of 100% (i.e., undiluted effluent) would yield a TUc of 1.0). In order for a test trial to pass, chronic toxicity of discharged ballast must not exceed 1.6 TUc for any species tested (equivalent to an EC25 of 62.5%). Calculation of a TUc greater than 1.6 for any test species will constitute a test trial failure based on residual toxicity within discharged ballast water.

Toxicity Quality Assurance:

Toxicity test acceptability (i.e., performance) criteria are presented in Tables 4 through 8. The quality assurance procedures for the algae tests will follow those discussed in detail in Section 13 of ASTM Designation E 1218-04 "*Standard Guide for Conducting Static Toxicity Tests with Microalgae*" (ASTM, 2006). Any deviations from the quality assurance procedures will be given in the final report.

GSI - The GSI's toxicity testing is designed to meet Section 5.2 of the Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9) as resolved by the Marine Environmental Protection Committee of the International Maritime Organization (IMO, 2008). Section 5.2 states that, "The advantage of conducting toxicity testing on the ballast water discharge is that it integrates and addresses the potential for interactions of the Active Substances and Preparations with the possible by-products." This section requires that, "these toxicity tests should include chronic test methods with multiple test species (a plant, an invertebrate and a fish) that address the sensitive life-stage. The preference is to include both a sub-lethal endpoint (growth or reproduction) and a survival endpoint." The following work plan will outline proposed methods to meet these IMO guidelines, using standard operating procedures (Table 3) developed by GSI which are based on methods approved by the USEPA (2002) and the American Society for Testing and Materials (ASTM, 2005).

Table 3. Great Ships Initiative Standard Operating Procedures Relative to Toxicity Testing.

GSI SOP Code	Test Type	Test Species	Test Endpoint
GSI/SOP/BS/RA/RT/5	Acute	*Selenastrum capricornotum*	Survival
GSI/SOP/BS/RA/RT/1	Acute	*Ceriodaphnia dubia*	Survival
GSI/SOP/BS/RA/RT/2	Acute	*Pimephales promelas*	Survival
GSI/SOP/BS/RA/RT/8	Chronic	*Selenastrum capricornotum*	Growth
GSI/SOP/BS/RA/RT/6	Chronic	*Ceriodaphnia dubia*	Reproduction
GSI/SOP/BS/RA/RT/7	Chronic	*Pimephales promelas*	Growth

Analytical Methods of Chlorine Determination:

Samples for chlorine analysis will be collected from the discharged water. Twenty ml of sample will be transferred from the sample collection container into a 30 ml beaker. The samples will have 200 µl of potassium iodide reagent and 200 µl of acetate buffer reagent added to them. Samples will be analyzed for total residual chlorine concentration as soon as possible after having been collected. Analysis will be conducted with a Thermo Orion Model 97-70 Residual Chlorine Electrode connected to an Orion Model 290A pH/mV/ISE meter.

A 100 mg/L chlorine stock solution will be prepared daily. Analytical standards, ranging in concentration from 5 to 3000 µg/l, will be prepared in deoxygenated deionized water by making dilutions of the 100 mg/l chlorine stock. Potassium iodide reagent and acetate buffer are added to the chlorine containing analytical standards. Chlorine present in the standards/samples oxidizes iodide to iodine in an acidic solution. The iodine concentration after the reaction is equal to the chlorine concentration present before the reaction. A calibration curve plotting log of the chlorine concentration (x-axis) versus the mV response from the Residual Chlorine Electrode (y-axis) is used to determine total residual chlorine concentrations in the samples.

Quality control sample analysis consists of analyzing duplicate samples and samples spiked with known amounts of chlorine. Approximately 10% of the samples will be analyzed in duplicate. This is also true for spiked samples in dechlorinated laboratory water.

Experimental Design and Test Conditions:

Toxicity tests will be conducted on the discharge from all test trials. As required by the IMO, the discharges will be tested with three freshwater species as described in Section 2.1 (IMO, 2006). Both acute and chronic data will be generated for each test. A dilution series, using Duluth-Superior Harbor water, will be run for each species.

Test samples will be collected at the time of discharge from the GSI facility. Samples will be collected by the GSI staff for analysis of both the efficacy of treatment at eliminating organisms from the ballast water and to investigate residual toxicity at discharge as described earlier in this document. Test water will be stored in large HDPE containers and held at 4°C in the dark to retain as much of the initial toxicity as possible. Portions of this sample will be used each day to serve as the renewal water for the bioassay. Sub-samples of each will also be sent to a certified chemistry laboratory for analysis of disinfection byproducts. GSI Testing Team will collect/ship all samples for chemical analysis and manage analytical results but costs of chemical analysis will be covered by Siemens.

All of the tests will be conducted in temperature-controlled incubators at the University of Wisconsin-Superior's Lake Superior Research Institute, which is located less than 6 city blocks from the testing facility immediately following sample collection.

Cold Water Effects:

One additional laboratory assay will be conducted to evaluate the toxicity and degradation of discharge water to simulate cold water treatment in Duluth-Superior Harbor. A 50 l subsample of treated water will be collected just downstream of the treatment system during one of the test trials, upon initial filling of the test tank. This subsample will then be placed in a dark, temperature controlled room at 4°C for the required 5-day holding time and then analyzed as described above, with the only modification being all assays will be conducted at 4°C.

Statistical Analysis:

Data will be analyzed using the SigmaStat® program (Jandel Corporation, 1995) and final results will be verified by using the TOXCALC® 5.0 program. Data analyses will included: normality, homogeneity of variance, one-way analysis of variance (ANOVA), and suite of tests for comparison between treatment means. Non-normal survival data will be transformed using the natural log (EPA 2000) to normalize the data. The endpoints of the dose-effectiveness experiments were the lethal concentration that provided 50 percent mortality, the lowest observed-effect-concentration (LOEC), and no-observed-effect-concentration (NOEC) of the test species except for the microbes. The LOEC is the lowest concentration in a test with a statistically significant response that is different from the control response. The NOEC is the highest test concentration for which there was no statistically significant difference from the control response. These measures are extrapolations of statistical results to the experimental endpoints. Mean percent survival and mean dry weight values for the laboratory controls and treatments will be analyzed with a statistical significance level of 0.05.

Quality Assurance/Quality Control:

Toxicity tests will be initiated with healthy, vigorous animals. Reference toxicant tests will be performed with the test species prior to the start of the definitive test. In the toxicity tests, percent survival and dry weights of survivors in the controls will be compared to published test acceptability criteria (U.S. EPA 2000) to determine the overall performance of the animals and the test system. Class I standardized weights are used as a check for the organism drying process and the performance of the balance. Daily and weekly calibration of test meters ensures optimal performance. Reference standards and duplicate samples will be used in the analysis of chlorine. The QC/QA documentation will be noted on the raw data sheets and study logbooks.

7. Evaluation Schedule (planned dates based on current plan and may vary)

- Test Plan for SiCURE finalized and Evaluation Agreements signed by May 22, 2009
- SiCURE ballast water management system delivered to *Cape Washington* for testing at MERC May 19, 2009
- SiCURE system installed and operating on the *Cape Washington* by May 29, 2009
- Two SiCURE calibration run completed by June 1, 2009
- MERC evaluation of the SiCURE systems initiated by June 15, 2009
- MERC will complete sample analysis and compile data from the evolution by July 31 2009
- SiCURE ballast water management system delivered to Superior, WI for testing at GSI by August 7, 2009

- SiCURE system installed and operating at GSI by August 17, 2009
- Two SiCURE calibration run completed by August 28, 2009
- GSI evaluation of the SiCURE system initiated by August 31, 2009
- GSI evaluation of the SiCURE system complete by October 9, 2009
- Draft report on the performance of the SiCURE system for review by the Advisory Board/Committee and Siemens by December 31 2009
- Final report submitted and released to public by March 2010

8. Data Recording, Processing, and Storage

This section describes methods employed during data recording, processing, and storage to minimize errors and assure high quality analyses.

MERC -

Documentation and Records:

A variety of data will be acquired and recorded electronically and manually by MERC partners (CBL/UMCES, SERC, UM and WREC) during this evaluation. Operational information and results will generally be documented in field/laboratory record books and on the data sheet/chain-of-custody forms (see below). Copies of these raw data will be transferred to the MERC office, which will store it permanently along with the rest of the study data.

Data Review:

All data are to be recorded directly in the field/laboratory record book as soon as they are available. Records are to be written in water-proof ink and written legibly. Any corrections will be initialed by the person performing the correction, will be crossed out with a line (not blackened or white-out), and will be dated according to the date that the correction was made. These data will include electronic data, entries in field/laboratory record books, operating data from the MERC test facility, and equipment calibration records. Records will be spot-checked within two weeks of the measurement to ensure that the data are recorded correctly. The checker shall not be the individual who originally entered the data. Data entries shall be checked in general for obvious errors and a minimum of 10 percent of all records shall be checked in detail. Errors detected in this manner shall be corrected immediately. The person performing the review will add his/her initials and the date to a hard copy of the record being reviewed. The MERC staff member will place this hard copy in the files for this evaluation. In addition, data generated by each MERC staff will be provided to the MERC Program Coordinator and reviewed before they are used to calculate, evaluate, or report results.

GSI -

Data Recording:

Specific forms (i.e., by size class of organism, by scale of testing, etc.) are used to record sample collection and analysis data. All relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of sample collection and analysis. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QAQC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QAQC officers and

checked for compliance. Problems identified by spot-checks are documented and included in a training/response file.

Specific forms (i.e., one for the GSI RDTE facility and one for bench-scale research activities) are also used to record sample custody, handling and storage information. Relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of changes to sample custody, and sample handling and storage. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QAQC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QAQC officers. Problems identified by spotchecks are documented and included in a training/response file.

Data Processing and Storage:

A database designed using the Microsoft Access software suite is used to store, manage and process data produced by the GSI. Microsoft Excel is used in conjunction with the database to create various dataset formats for subsequent analysis. Database entry and maintenance is the responsibility of the GSI database management staff. Regular checks for data entry errors are conducted by comparing database records with the original paper data sheets. This is a manual inspection process and though rather time consuming, it is an essential procedure for discovering errors. After examination and QA analysis, the data distribution files from the Access database are posted to the Lake Superior Research Institute's (LSRI's) Local Area Network (LAN) in an organized hierarchical folder system such that those relevant GSI personnel are able to access the data. A backup of the database is also made regularly to avoid any loss of data following computer/electronic glitches. Files are also posted to the GSI's sharepoint website such that those GSI personnel outside of the LSRI network can access the data. Posting to sharepoint also acts as a secondary data backup/storage mechanism.

9. Quality Assurance/Quality Control

MERC -

Treatment performance evaluations are implemented according to the Test/QA plans and technical documents (e.g., Standard Operating Procedures) prepared during planning of the evaluation. Prescribed procedures and a sequence for the work are defined during the planning stages, and work performed shall follow those procedures and sequence. Technical procedures shall include methods to assure proper handling and care of test instruments. All implementation activities are documented and are traceable to the Test/QA plan and SOPs and to test personnel.

Analytical Laboratory Quality Control:

The analyses for Chlorophyll, TSS and POC shall have the following Quality Controls:

a. Blanks

Three times during the evaluation, analysis of blanks. These blanks will be collected weekly during sampling and should include Field Blanks (see Section 7.4.2).

b. Control Charts. Two types of control charts are used in laboratories: a mean chart for blanks and a range chart for replicate analyses.

Quality Control for Instrument Calibration:

The test instrumentation to be used in the evaluation will be calibrated by the MERC staff according to the SOPs for the instrumentation prior to use. A calibration log will be created for each instrument. The logs shall include at least the following information: name of instrument, serial number and/or identification number of instrument, date of calibration, and calibration results. These logs shall be provided to the MERC Program Coordinator and maintained in a master calibration file as part of the QA/QC records.

Laboratory Test Quality Control:

All analytical measurements are performed using materials and/or processes that are traceable to a Standard Reference Material. Standard Operating Procedures are utilized to trace all quantitative and qualitative determinations to certified reference materials. All metrology equipment (analytical balances, thermometers, etc.) is calibrated using materials traceable to the National Institute of Standards and Technology (NIST) and maintained on a schedule to ensure accuracy.

All volumetric glassware must be calibrated as conforming to Class A. A valid certificate of calibration or compliance must be available for each item. If the item has been calibrated in-house, the laboratory shall have a documented record of the calibration data showing traceability to national standards. Since the capacity of volumetric glassware may change with use, the calibration should be verified at regular intervals. Volumetric capacity is normally determined gravimetrically, using water conforming to the MERC glassware calibration Standard Operating Procedure (SOP). Before starting, care will be taken to ensure that the glassware is clean.

Field Logs:

Standard uniform field logs will be maintained for the evaluation. These logs should report name of staff conducting fieldwork, date (month, day, and year), operating status of all equipment, and manual readings of environmental conditions.

Field Quality Control Samples:

Field quality control samples provide information on the potential for bias due to contamination of analytical results by sample collection, processing, shipping, and analysis. To ensure that the field sample collection and analysis procedures are properly controlled, field blanks and replicate samples will be taken three times during the evaluation. These will be analyzed in the same manner as the collected samples for Chlorophyll, TSS, and POC. Field blanks are generated under actual field conditions and will account for all sources of contamination that might be introduced to a sample including incidental or accidental sample contamination during the entire process of sampling, transport, sample preparation, and processing. While field blanks mimic sample collection and processing, they do not come in contact with ambient water.

Sample Custody:

All samples will be accompanied by the sample collection sheet and a Chain-of-Custody (COC) form.

The COC specifies time, date, sample location, unique sample number, requested analyses, sampler name, required turnaround time, time and date of transaction between field and laboratory staff, and name of receiving party at the laboratory. Proper labeling of sample bottles

is critical. The COC is a mechanism by which a sample can be tracked through the various phases of the process: collection, shipping, receiving, logging, sample prep/extraction, analysis, and final data QA/QC review.

When transferring the possession of the samples, the transferee must sign and record the date and time on the chain-of-custody record. Custody transfers, if made to a sample custodian in the field, should account for each individual sample, although samples may be transferred as a group. Every person who takes custody must fill in the appropriate section of the chain-of-custody record. The MERC staff member is responsible for properly packaging and dispatching samples to the laboratory for analysis. This responsibility includes filling out, dating, and signing the appropriate portion of the chain-of-custody record. The original and one copy of the chain-of-custody record form should be placed in a plastic bag inside the secured shipping container with the samples. One copy of the chain-of-custody record form should be retained by the MERC staff member at each MERC partner institution. The transportation case should then be sealed and labeled. All records should be filled out legibly in waterproof pen.

Sample Handling:

All collected physical, chemical, and biological samples will be handled in the same manner. Each sample will be dated and coded according to the appropriate sample sequence. The actual sample container will be labeled with a number for identification. Samples stored for any period of time shall be routinely inspected by the MERC staff member to assure proper preservation and label integrity. The storage containers and storage devices (e.g., freezers and locker) must be inspected routinely for proper operation and integrity. Results of all inspections shall be included in the sample records. All logs shall be duplicated weekly. The original shall be retained at the MERC partner site and a copy shall be sent to the MERC Program Coordinator.

Audits:

MERC Program Coordinator will perform a technical systems audit twice during the evaluation. The purpose of this audit is to ensure that the tests are being performed in accordance with the MERC Protocols, published reference methods, and any SOPs used. In this audit, the MERC Program Coordinator may review the reference methods used, compare actual test procedures to those specified or referenced in the Protocols, and review data acquisition and handling procedures. A technical systems audit report will be prepared, including a statement of findings and the actions taken to address any adverse findings.

MERC Program Coordinator will also audit approximately 10% of the evaluation data acquired during the tests to determine if data have been collected in accordance to the Protocols with respect to compliance, correctness, consistency, and completeness. The MERC Program Coordinator will trace the data from initial acquisition to final reporting.

Finally, each assessment and audit will be documented, and assessment reports will include the following:

a. Identification of any adverse findings or potential problems,
b. Response to adverse findings or potential problems,
c. Possible recommendations for resolving problems,
d. Citation of any noteworthy practices that may be of use to others, and
e. Confirmation that solutions have been implemented and are effective.

Corrective Action:

The MERC Program Coordinator, during the course of any assessment or audit, will identify to the MERC staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the MERC Program Coordinator will consult with MERC Primary Investigators and is authorized to stop work. Once the assessment report has been prepared, the MERC Program Coordinator will ensure that a response is provided for each adverse finding or potential problem and will implement any necessary follow-up corrective action. The MERC Program Coordinator will ensure that follow-up corrective action has been taken.

QA/QC Document Control:

It is the responsibility of the MERC Program Coordinator to maintain QA/QC records, which shall include the following:

1) records of the disposition of samples and data.
2) records of calibration of instruments.
3) records of QA/QC activities, including audits and corrective actions.

GSI -

Quality Assurance Project Plan (QAPP):

The GSI's Quality Assurance Project Plan (QAPP) outlines the management activities the GSI undertakes to assure the credibility of its biological research activities. The plan covers QA/QC data quality indicators, evaluation processes, performance measures and acceptance criteria; instrument certification and calibration; personnel training requirements; QA/QC of documents and records; data management; and QA/QC assessments and response actions; etc. The plan is updated annually, with a specific process used for review, comment, approval, distribution and posting. It closely follows the format of the U.S. Environmental Protection Agency's (EPA's) "*EPA Guidance for Quality Assurance Plans*".

Quality Assurance/Quality Control Activities:

GSI QA/QC Officers regularly observe all aspects of the GSI's biological research activities and conduct technical reviews of these activities to ensure that all information is complete, that SOPs are correctly followed, and that QA/QC objectives are met. The results of these observations are documented on a QA/QC audit report form. It is the responsibility of the GSI QAQC Officer undertaking the audit to maintain the form on file, create an electronic copy, and post to the GSI Sharepoint website for storage. The GSI Senior QA/QC Officer also uses these audits to help prepare an annual report describing all GSI QA/QC activities, including results of QA/QC observations, corrective actions, etc. Corrective action reports help resolve any identified deficiencies and non-compliance issues that relate to on-going activities and problems of a systematic nature.

Standard Operating Procedures (SOPs):

GSI SOPs are developed by the relevant GSI senior personnel in conjunction with the PI and GSI Senior QA/QC Officer. The SOPs follow a common format and all include specific QA/QC procedures and metrics. The Senior QA/QC Officer is responsible for distributing the

SOPs to the relevant parties for approval. Draft and final copies of all SOPs are posted to the GSI Sharepoint website; the final version is also posted to the GSI public website. SOPs are updated on an as-needed basis.

Documents and Records:

The GSI Senior QA/QC Officer is responsible for maintaining all documents and records for a period of ten years unless custody is transferred using a chain of custody form. Electronic versions of all GSI documents and records are saved to the GSI Sharepoint website once complete. Hard copies of GSI documents and records are scanned and also saved to the GSI Sharepoint website. Due care and diligence will be taken to properly dispose of documents and records that are no longer required after a 10 year period has elapsed. Disposal procedures will involve electronic deletion of documents and records from the GSI Sharepoint website and the personal computers of GSI personnel, as well as manual shredding of hard copies.

Notebooks:

Bound field and laboratory notebooks are used to record observations, sampling details and on-site laboratory and field measurements. Notebooks are also used to record instrument and equipment calibration and maintenance information. All notebooks are examined periodically by the GSI QA/QC officers and checked for compliance with SOPs. Problems identified by the periodic QA/QC review will be documented and included in a training/response file.

Sample Collection and Analysis Records:

Specific forms (see individual SOPs) are used to record sample collection and analysis data. All relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of sample collection and analysis. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QA/QC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QA/QC officers. Problems identified by spot-checks are documented and included in a training/response file.

Sample Management Records:

Specific forms are used to record sample custody, handling and storage information. All relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of changes to sample custody, and sample handling and storage. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QA/QC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QA/QC officers. Problems identified by spot-checks are documented and included in a training/response file.

Safety, Operation and Maintenance Records:

All relevant GSI personnel particularly those involved with operating the GSI RDTE land-based facility are responsible for ensuring that all forms associated with safety, operation and maintenance (i.e., confined space entry permit forms) are correctly filled out. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QA/QC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QA/QC officers. Problems identified by spot-checks are documented and included in a training/response file.

10. Roles and Responsibilities

The evaluation is coordinated and supervised by the MERC and GSI Principal Investigator, Program Coordinator and MERC and GSI personnel. Staff participate in this test by installing, maintaining, and operating the respective technologies throughout the test; operating the reference equipment, collecting the water samples, downloading the data from the instrument package, and informing the MERC Program Coordinator staff of any problems encountered. Manufacturer representatives shall train MERC and GSI staff in the operation of their treatment system. However, the proper installation, calibration, maintenance, and operation of the systems is ultimately the responsibility of the manufacturer. QA oversight is provided by the MERC and GSI Program Coordinator. In addition to aiding the development of these protocols, the MERC and GSI Advisory Board/Committee will be consulted during the evaluation in the event problems occur, will assist in the analyses of results, and will review the final Treatment Performance Report prior to release. Specific responsibilities are detailed below.

The MERC and GSI Principal Investigators have the overall responsibility for ensuring that the technical goals and schedule established for the evaluation are met and the final authority on decisions regarding this evaluation. The Principal Investigators shall:

- Prepare the draft Test Protocols/QA Plan and Treatment Performance Evaluation.
- Revise the draft Test Protocols/QA Plan and Treatment Performance Evaluation in response to reviewers' comments.
- Finalize the Test Protocols/QA Plan and Agreement for this Treatment Performance Evaluation.
- Sign the Treatment Performance Evaluations Agreement on behalf of MERC and GSI.
- Aid in treatment system testing.
- Aid in the preparation of a final report on this Treatment Performance Evaluation.
- Provide final approval of the Treatment Performance Evaluation Report.

The Program Coordinators shall:

- Help prepare the draft Test Protocols/QA Plan and Treatment Performance Evaluations
- Help revise the draft Test Protocols/QA Plan and Treatment Performance Evaluations in response to reviewers' comments.
- Coordinate distribution of the final Test Protocols/QA Plan and Treatment Performance Evaluation.
- Coordinate testing, measurement parameters, and schedules.
- Ensure that all quality procedures specified in the test/QA plan are followed.
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.
- Serve as the primary point of contact for manufacturers and Testing Teams.
- Ensure that confidentiality of proprietary manufacturer technology and information is maintained.
- Review the draft Test Protocols/QA Plan and Treatment Performance Evaluations.
- Conduct a technical systems audit (TSA) once during the evaluation.
- Audit at least 10% of the verification data.
- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective action.

- Determine if a stop work order should be issued if audits indicate that data quality is being compromised or if proper safety practices are not followed.
- Provide a summary of the audit activities and results for the verification reports.
- Review the draft Evaluation reports.
- Have overall responsibility for ensuring that the test/QA plan, SOPs and QMP are followed.

Testing Teams* shall:

- Assist in developing the Test Protocols/QA Plan.
- Perform sample collections and analyses as detailed in the test procedures section of the test/QA plan.
- One member of the Testing Team will conduct 10% data audit as described in QA procedures. This will be done for all data logs and electronically entered data.
- Provide all test data to the Program Coordinator electronically, in mutually agreed upon format.
- Provide the Program Coordinator access to and /or copies of appropriate QA documentation of test equipment and procedures (e.g., SOPs, calibration data).
- Provide information regarding education and experience of each staff member involved in the evaluation.
- Assist in reporting of their respective test facility's QA/quality control results.
- Review portions of the draft Performance Evaluations to assure accurate descriptions of their respective test facility operations and to provide technical insight on evaluation results.

*MERC Testing Team includes researchers from the University of Maryland Center for Environmental Science, Smithsonian Environmental Research Center, University of Maryland at College Park, University of Maryland Wye Research and Education Center, and the crew of the *M/V Cape Washington*. A complete list, with qualifications, is available upon request.

* GSI Testing Team includes researchers from the University of Wisconsin Superior Lake Superior Research Center; University of Minnesota-Duluth Natural Resources Research Institute, and private consultants. A complete list, with qualifications, is available upon request.

Manufacturers shall:

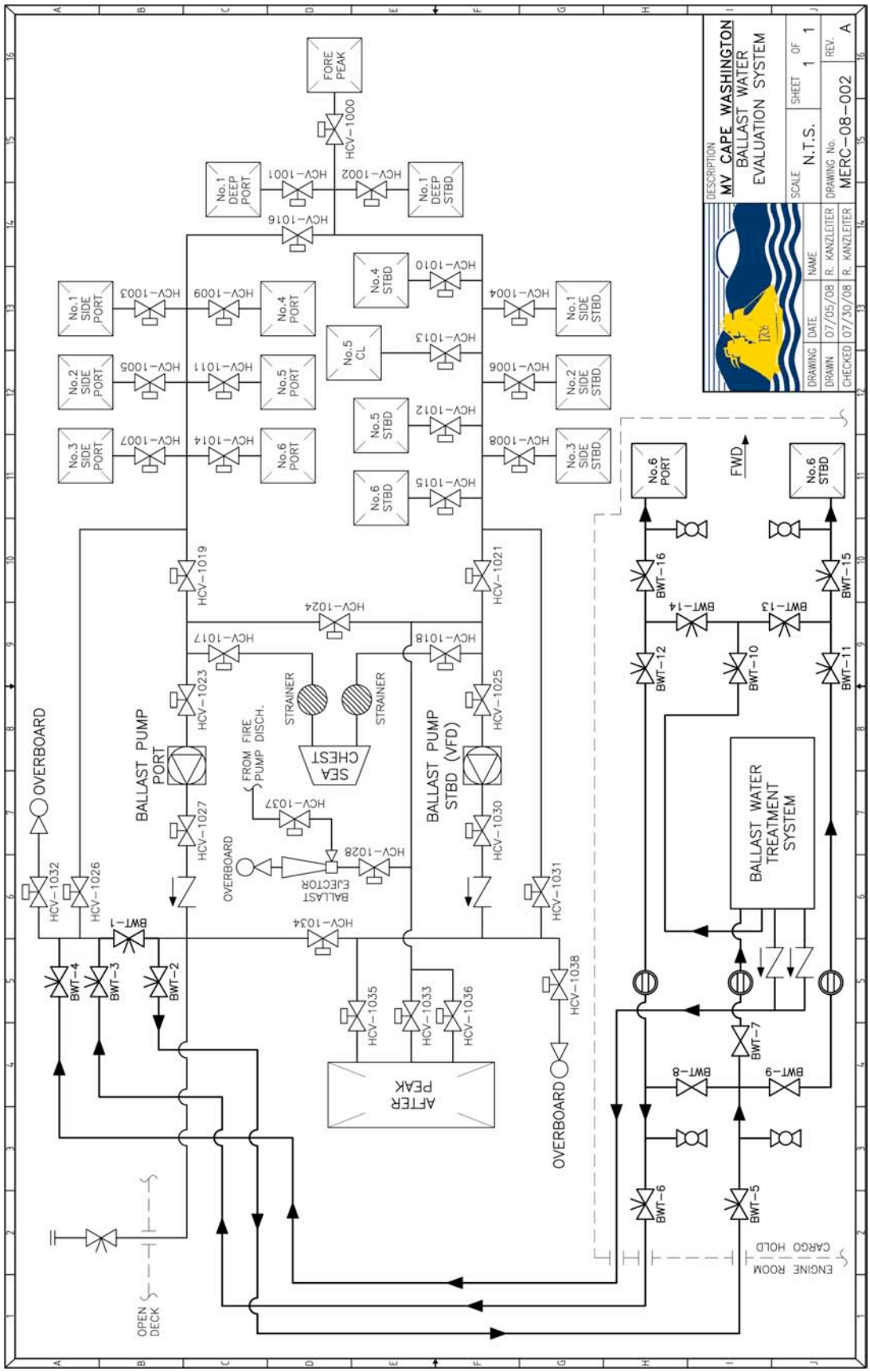
- Review the draft test/QA plan and provide comments and recommendations.
- Work with MERC to commit to a specific schedule for testing.
- Provide an operational treatment systems for the agreed upon test site.
- Aid in the installation, calibration and operation of treatment system for testing.
- Review and comment on draft Performance Report.

Advisory Board/Committee* shall:

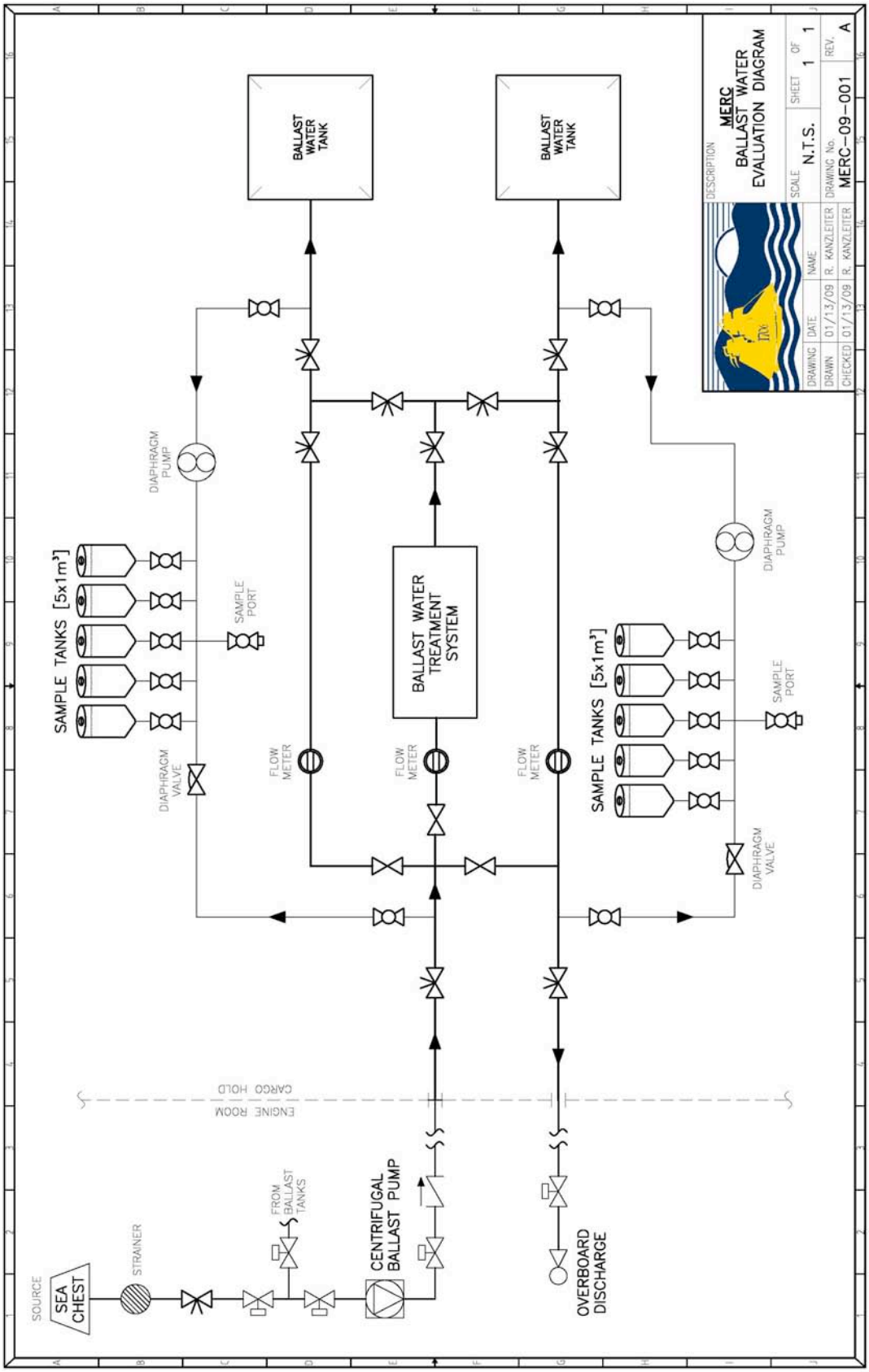
- Assist in developing the Test Protocols/QA Plan.
- Approve the final Test Protocols/QA Plan.
- Provide specific advice during testing.
- Review and comment upon draft Performance Report.

*A list of current MERC Advisory Board and GSI Advisory Committee members, and their affiliations, can be found at www.maritime-enviro.org and www.nemw.org/GSI/.

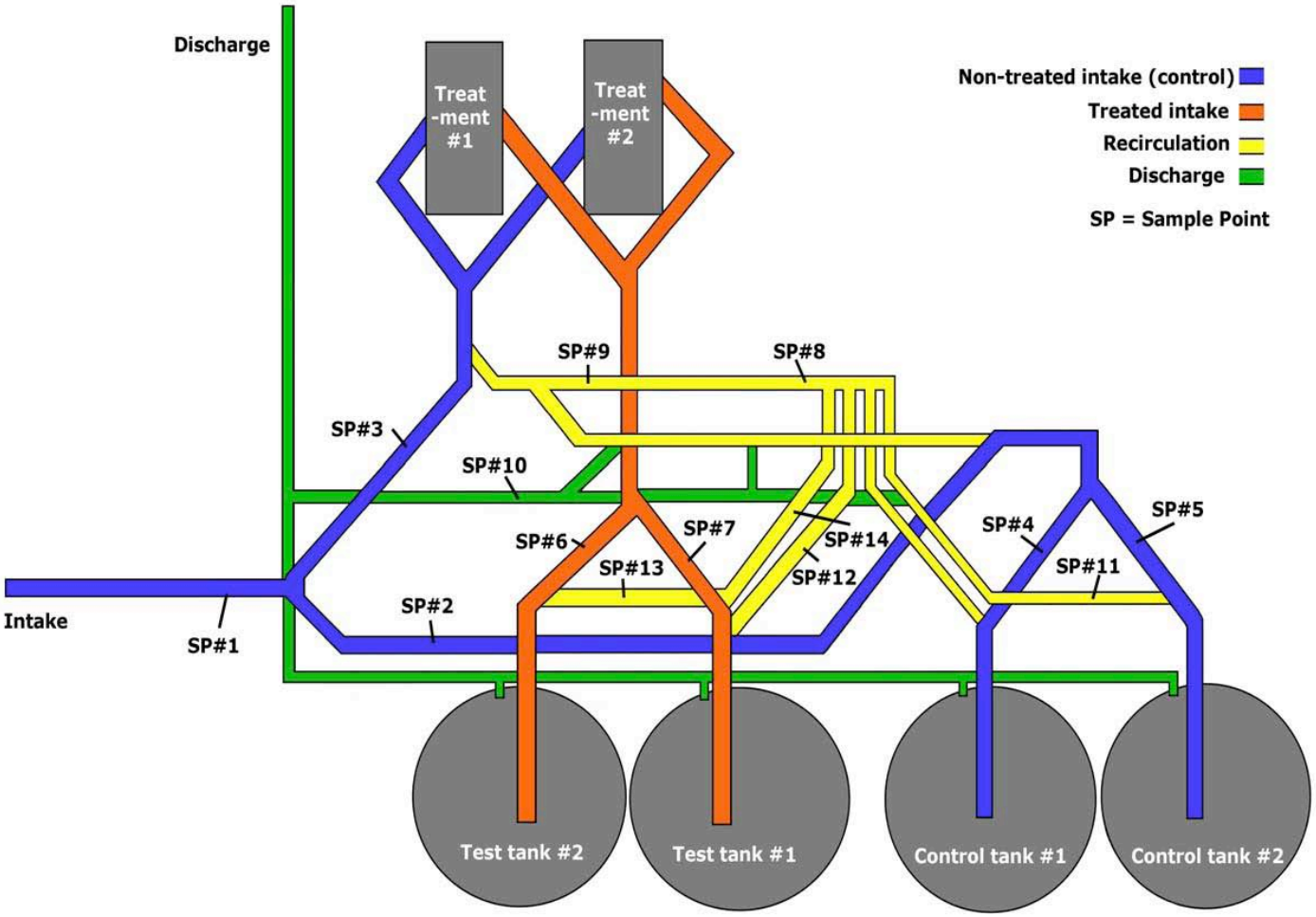
11. Modified *Cape Washington* ballast system to allow for treatment testing by MERC.



12. MERC *Cape Washington* test setup and sampling design.



13. Simplified schematic of the GSI land-based facility.
Detailed engineering drawings can be found at www.nemw.org/GSI/**.**



14. Table 2, Samples to be collected with corresponding volumes and purpose.

MERC and GSI will be collecting a variety of data on physical, chemical, biological, and toxicological parameters during this evaluation. In some case, values will be determined directly for the water using in situ sensors or instruments. Table 2 described the water sample that will be collected and analyzed for each time point and treatment of each test trial, excluding toxicity samples that are described above and in tables 3-8.

Parameter	Time Point / Treatment	Purpose	MERC volume	GSI volume
Total Suspended Solids (TSS)	Initial filling	Quantify challenge water	500 ml	500 ml
Particulate Organic Material (POC)	Initial filling	Quantify challenge water	500 ml	500 ml
Dissolved Organic Material (DOC)	Initial filling	Quantify challenge water	500 ml	500 ml
Zooplankton (> 50 μm) / m^3	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify live organisms > 50 μm in size	5 m^3	3 m^3
Phytoplankton (10 - 50 μm) / ml	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify live organisms 10 - 50 μm in size	10 l	3 l
Bacteria cfu / ml	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify microbial communities	5 l	3 l

15. Tables 4 – 8, Summaries of MERC Toxicity Test Methods

Table 4. Summary of the Test Conditions and Test Acceptability Criteria for the Sheepshead Minnow *Cyprinodon variegatus* 96-Hour Acute Toxicity Test

Test type:	Static renewal
Test duration:	96 h
Temperature:	25 ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	250 ml
Test solution volume:	200 ml
Renewal of test solutions:	After 48-h
Age of test organisms:	1 to 14 days; 24-h range in age
No. organisms per test chamber:	10
No. replicate chambers per concentration:	2
No. organisms per concentration:	20
Feeding regime:	*Artemia* nauplii (<24 h old) during holding; Feed approximately 0.2 ml Artemia nauplii concentrate 2 h prior to renewal at 48 h.
Test chamber cleaning:	Cleaning prior to 48 h renewal
Test chamber aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test dilutions:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution series:	0.56 dilution series
Endpoint:	Mortality
Test acceptability criterion:	90% or greater survival in controls

Table 5. Summary of Test Conditions and Test Acceptability Criteria for the Sheepshead Minnow *Cyprinodon variegatus* Larval Survival and Growth Chronic Test

Test type:	Static renewal
Test Duration:	7 d
Temperature:	25 ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	500 ml
Test solution volume:	250 ml
Renewal of test solutions:	Daily
Age of test organisms:	Newly hatched larvae <24 hours old
No. larvae per test chamber:	10
No. replicate chambers per concentration:	4
No. larvae per test concentration:	40
Feeding regime:	*Artemia* nauplii (<24 h old). On days 0-2, feed 0.10g wet weight newly hatched (<24 hours old) brine shrimp nauplii daily. On days 3-6, feed 0.15g wet weight newly hatched (<24 hours old) brine shrimp nauplii daily.
Cleaning:	Siphon daily, immediately before test solution renewal
Aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution factor:	0.56
Endpoint:	Survival and growth (dry weight)
Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chamber equals or exceeds 0.60 mg

Table 6. Summary of the Test Conditions and Test Acceptability Criteria for the Mysid *Americamysis bahia* 96-Hour Acute Toxicity Tests

Test type:	Static renewal
Test duration:	96 h
Temperature:	25 ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	250 ml
Test solution volume:	200 ml
Renewal of test solutions:	After 48-h
Age of test organisms:	1 to 5 days; 24-h range in age
No. organisms per test chamber:	10
No. replicate chambers per concentration:	2
No. organisms per concentration:	20
Feeding regime:	*Artemia* nauplii (<24 h old) during holding; Feed approximately 0.2 ml Artemia nauplii daily.
Test chamber cleaning:	Cleaning prior to 48 h renewal
Test chamber aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution series:	0.56 dilution series
Endpoint:	Mortality
Test acceptability criterion:	90% or greater survival in controls

Table 7. Summary of Test Conditions and Test Acceptability Criteria for the Mysid *Americamysis bahia* Larval Survival and Growth Chronic Test

Test type:	Static renewal
Test Duration:	7 d
Temperature:	26ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	400 ml
Test solution volume:	150 ml
Renewal of test solutions:	Daily
Age of test organisms:	7 d
No. organisms per test chamber:	5
No. replicate chambers per concentration:	8
No. organisms per test concentration:	40
Feeding regime:	Feed 150 <24 h old *Artemia* nauplii daily, half after test solution renewal and half after 8-12 h.
Cleaning:	Siphon daily immediately before test solution renewal and feeding.
Aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution factor:	0.56 dilution series
Endpoint:	Survival and growth (dry weight)
Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chamber equals or exceeds 0.20 mg; fecundity may be used if 50% or more of females in controls produce eggs.

Table 8. Summary of Test Conditions and Test Acceptability Criteria for the Algae *Isochrysis galbana* and *Tetraselmiss uecica* Chronic Growth Test

Test type:	Static non-renewal (required)
Temperature:	20 ºC ± 1 ºC
Light quality	"Cool white" fluorescent lighting (recommended)
Light intensity:	360-440 foot candles
Photoperiod:	Continuous illumination
Test chamber size:	250 ml
Test solution volume:	100 ml
No. replicate chambers per concentration:	4
Renewal of test solutions:	None
Age of test organisms:	Log growth phase
Initial cell density in test chambers:	1-2 X 10^4 cells/ml
Shaking rate:	100 rpm continuous on a mechanical shaker or twice a day hand shaken
Aeration:	None
Nutrient solution	Algal assay culture medium nutrients added to each replicate (Appendix A3 of ASTM Designation E 1218-04; ASTM, 2006)
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution factor:	0.56 dilution series
Test duration:	96 hours
Endpoint:	Growth (cell counts)
Test acceptability criterion:	Mean cell density of at least 1 x 10^6 cells/ml in the controls; and variability (CV%) among control replicates less than or equal to 20%